VARIATIONS OF THE ULTRASTRUCTURE OF *PROTOTHECA WICKERHAMII* (CHLOROCOCCALES, CHLOROPHYCEAE) PLASTID ACCORDING TO THE CARBON NUTRITION

Françoise PUEL[1] [2] and Georges GIRAUD[1]

(1) Laboratoire des biomembranes et surfaces cellulaires végétales, UA CNRS 311, ENS, 46 rue d'Ulm, 75231 Paris, France.
(2) Laboratoire de Parasitologie, Faculté de Médecine de Créteil. 6 rue du Général Sarrail, 94010 Créteil, France.

ABSTRACT - Two morphologically different strains of *Prototheca wickerhamii* were studied with different sources of carbon compounds. The two strains were able to use glucose and glycerol, but with mannose, hexadecane and pentane the growth was slow and the store substances were rare or absent. Thus the diversity of the plastid structure is revealed that permits comparison with other algae and knowledge of its functional potentialities. In certain cases microtubule-like and paracristalline structures appeared: their significance is discussed.

RÉSUMÉ - L'assimilation de sources de carbone variées a été étudiée pour deux souches de *Prototheca wickerhamii* différant par leurs caractères morphologiques. Les deux souches utilisent le glucose et le glycérol, mais avec le mannose, l'hexadecane et le pentane leur croissance est lente et leurs réserves sont rares ou absentes. Ces variations dans le métabolisme ont permis de mettre en évidence la variabilité de la structure du plaste, de le comparer à celui d'autres algues et de comprendre son fonctionnement. Dans certains cas des microtubules et des structures paracristallines apparaissent: leur signification est discutée.

KEY WORDS : Parasitic alga, *Prototheca wickerhamii,* plastid ultrastructure, carbon starvation, protein stores.

INTRODUCTION

Whereas physiological studies on *Prototheca* are being made in a large number of laboratories, very little cytological research has been made. This is probably due to the difficulty experienced in fixing the cells which contain a resistant biopolymer "sporopollenin - like" (Puel *et al.*, 1987) and to the diameter of the cells (about 10μm). The genus *Prototheca* comprises four species (Pore, 1985): *Prototheca zopfii* Krüg., *P. wickerhamii* Tubaki et Soneda,

P. moriformis Krüg. and *P. stagnora* Cooke but most of the studies have been made on *Prototheca zopfii*. For many years we have been using a strain of *Prototheca wickerhamii* for cytologicall research. This alga grows either on a Sabouraud's medium or an Anderson's medium but on these media rich in carbon and nitrogen, the cells appear to be full of starch granules and various storage granules and it is difficult to locate or even to identify cellular organelles. It seemed interesting to try to modify these culture media by modifying the source of carbon. Thus we could obtain changes in the metabolism of the stores and nutritional shortages as well. Variations of cellular content thus obtained would enable a better observation of organelles. During these experiments we noticed that the plastid morphology varied with changes in carbon source but it still showed many of its functional abilities. It was decided to study these changes. Two morphologically different strains of *P. wickerhamii* were subjected to the same treatment in order to check that the physiological and cytological reactions were similar in both cases, particularly as regards their plastids. In this work we have laid less emphasis on the problem of algae permeability to sugars and on the study of their assimilation in order to classify them and more emphasis on finding the growth conditions which make it possible to compare the fine structure of the organelles whose shapes were distorted by starvation to those of the algae growing on an optimum medium. Attempts were made to describe, in the most propitious conditions, the characteristics of the colourless plastids and to check the persistance of some of their functions.

MATERIALS AND METHODS

The two strains of *P. wickerhamii* which we used were from the culture collection of the Institut Pasteur of Paris (strains 1094-74 and 1202-79). They were grown for two weeks on agar plates incubed at room temperature and in light. The utilization of organic carbon sources was studied in both Sabouraud's and Anderson's media culture (Patni *et al.*, 1974). This last medium has also been used by Pore (1985) who has slightly modified the mineral salts composition and by Kessler (1982) who has tested the organic carbon assimilation of *Prototheca*. The glucose and glycerol of these media were replaced by lactose, saccharose, maltose, mannose at a concentration of 8 g/l or by pentane and hexadecane at a concentration of 1%. These organic carbon compounds were selected so as to compare our results with those of authors who have used them either to classify them or to observe their metabolic pathway (Kessler, 1982; Pore, 1985). We have used also pentane and hexadecane as many authors (Koenig, 1983, 1984; Pore, 1983; Walker *et al.*, 1975, 1978) have studied the hydrocarbon degradation by the algae of the *Prototheca* genus. Sabouraud's and Anderson's media without organic carbon source have been designated S° and A°. Cells were prepared for electron microscopy by technique previously described (Puel *et al.*, 1982) and photomicrogra en with the Philips 400 electron microscope.

RESULTS

According to the medium used the appearance of the plastid varies. Both strains of *P. wickerhamii* are able to utilize glucose and glycerol but not many other sources of carbon for growth, according to Kessler's results (1982). The culture medium S° supports the growth of both strains with non-utilisable sugars. We found that peptone used without organic carbon compounds can serve as a source of nitrogen and carbon as well. As the energy output is weak, growth is reduced and no storage granules can be observed.

The culture medium A° without organic compound (ammonium chlorid was used as the source of nitrogen) cannot support the growth of either strain of *Prototheca*. Thus this medium makes it possible to test the penetration of organic carbon into the algae.

The two strains grew well with glucose and glycerol and more slowly with mannose. On the other hand their behaviour differs with hydrocarbons: strain 1 grew slowly while strain 2 grew relatively well on hexadecane. With pentane strain 1 grew slowly and strain 2 did not develop.These variations in the development of the cells provoked the following cytological modifications.

1. The plastid of *Prototheca* in Anderson's medium

A. Plastid with starch

Strain 1 with glycerol and mannose.

Strain 2 with glycerol, mannose and hexadecane.

In Anderson's medium with glycerol the plastid is identified by the starch granules which fill it. On each micrograph there are many profiles of the organelle which mean that either there are many plastids or that the plastid has many lobes. The stroma may be seen between the starch granules, it contains stratified lamellar structures more or less joined (Fig. 1). These recall the "microtubule-like" structures described by Pickett-Heaps (1968) in algae chloroplastids or by Lawrence (1984) in spinach plastids. The two strains show similar appearence. In culture medium A° with mannose the growth of the two strains differ from one another but the cells have the same appearance. The number of starch granules decreases and the plastid becomes very deformed, it lengthens and swells at the ends where several starch granules can be seen. Here again microtubule-like structures are to be found in the stroma between the starch granules (Fig. 3). In A° supplemented by hexadecane, only the strain 2 grows relatively well and produces starch granules (Fig. 5).

B. Plastid without starch

Strain 1 with hexadecane and pentane

With hexadecane or pentane strain 1 grows slowly, its plastid lengthens and swells at both ends and becomes tubular in the middle, it contains no starch granules (Figs. 2 et 4).

2. The plastid of *Prototheca* in Sabouraud's medium

Micrographs of thin sections from algae cultivated in Sabouraud's medium show the presence of plastids full of starch granules similar to those from algae cultivated in Anderson's medium. In S° medium the plastids are more deformed than in the previous cases, the swollen parts with irregular sizes never contains starch granules and are connected by long and twisted peduncles. In the stroma there are dense paracristalline formations either as a network or in flake, which look like protein storage accumulations, their presence is constant in the plastids. Accumulation occurs in the medium of the swollen portions (Figs. 7, 8, 9, 10, 11). The microtubule-like structures are seen again (Figs. 7, 9, 11).

DISCUSSION

Anderson's and Sabouraud's media have been designed for different purposes. The first may be considered as derived from a medium used for photosynthetic algae in heterotrophic conditions (*Chlorella* for example). The second is derived from a medium for strict heterotrophic organisms (fungi or bacteria), and in particular the source of nitrogen is an organic compound: peptone. On these media with easily digested sugars, the quick growth always is associated with prominent starch granule accumulation in the plastid of *Prototheca* which becomes obscured and the stroma is completely concealed. The sources of organic compounds, supporting a weaker growth (mannose, pentane, hexadecane), make for a better observation of the plastidial profiles and the cellular organelles possible. When the stroma is seen, paracristalline formations are found; they recall the structures of protein stores which are often seen in the store cells of angiosperm seeds (Lemoine, 1966). These structures are not really microtubule-like but form complex paracristalline structures. These formations are characteristic of the cells which grow in S° medium. The peptone in the medium causes a rather reduced carbon nutrition which does not produce the starch synthesis, but an accumulation of amino acids and they form these figures. Thus the plastid maintains a double function: it acts as starch store which proves the presence of functional enzymatic systems in it but it may also serve as protein stores. This function is also to be found in the proplastids of the higher plants (Buvat, 1963) and in the red algae (Giraud *et al.*, 1983).

Newcomb (1967) finds, in addition to the formations described above, microtubular profiles which he compares to the prolamellar body that has been seen in some algae (Pickett-Heaps, 1968). On the other hand many authors (Rivera, 1982; Lawrence, 1984) have described amoeboid plastids containing microtubule-like structures. This aspect is sometimes considered as juvenile. The structures observed here may be thought to be microtubule-like particularly the peripherical microtubules. Then, the carbon starvation could be said to cause a regression of these plastids towards a state similar to proplastids. The stretched appearance with swollen ends exists in many higher plants in deficiency conditions particularly in mutants (Sprey *et al.*, 1965, 1966; Valanne *et al.*, 1972). One may think either that the plastid has some difficulty in dividing and thus forms diverticules or that the absence of

starch synthesis and renewal of stroma cause a collapse in the plastids which still conserves a big plastidial membrane. We have noticed that these cytological modifications are reversible.

Our observations about the utilization of different carbon sources for the growth of these two strains differ from those of some authors. Kessler (1982) finds that mannose does not support the growth of *P. wickerhamii* and Walker (1978) and Pore (1983) state an absence of n-alkane metabolisation. Differences in the growth and appearance between two strains have already been observed (Kessler, 1982; Pore, 1985), they are the results of the heterogeneity of the organism without sexual reproduction and they make it difficult to generalise about the phylogenetic relationships using the studied characteristics.

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LÉGENDES DES PLANCHES

Fig. 1: Plastid of a cell grown in Anderson's medium. The plastid is full of starch granules. The stroma contains stratified lamellar structures (arrows). (Scale bar: 0,2μm). - Fig. 3: Plastid of a cell grown in A° medium with mannose. The plastid becomes very deformed, several starch granules are seen. In the stroma microtubule-like structures are to be found (arrow). (Scale bar: 0,1μm). - Fig. 2 and 5: Plastids of strains 1 and 2 grown in A° medium with hexadecane. The plastids of the strain 2 are not deformed and contains starch granules (Fig. 5). These of strain 1 are tubular in the middle (arrow) and swollen at the two ends, they do not contain starch granules (Fig. 2). (Scale bar: 0,2μm). - Fig. 4: Plastid of a strain 1 grown in A° medium with pentane. The plastid is lengthened and does not contain starch granules (arrow). (Scale bar: 0,2μm).

Figs 6, 7, 8, 9, 10: Plastids of the strains 1 and 2 in S° medium. The plastids are very deformed, the swollen portions with irregular sizes do not contain starch granules and are connected by long and twisted peduncles. In the stroma there are dense paracristalline formations; their appearance is different, due to the orientation of the sections. The microtubule-like structures are also seen again (arrow). (Scale bar: 0,2μm).

Figs 1, 2, 3, 4, 5, 6, 7, 8, 9, 10: Fixation glutaraldehyde, osmium tetroxide, uranyl acetate. Staining: uranyl acetate, lead.